Determination of the potential of 5-Hydroxy-L-tryptophan and L-tryptophan as therapeutic agents for prostate cancer

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Abstract

Prostate cancer is the second leading cause of death from cancer in men. Androgen deprivation therapy (ADT) is used as the standard treatment in prostate cancer, and this treatment has undesirable side effects over time. There is a need for more effective, safe compounds that occur naturally under the influence of these undesirable limitations. In this study, the anti-cancer potentials of 5-Hydroxy-L-tryptophan (5-HTP) and L-tryptophan, which are thought to have various inhibitory potential on cancer and its mechanisms, were studied. The study results showed that 5-HTP has a significant inhibition in prostate cancer cells. This study is an important preliminary screening for new and effective molecule trials. In order to develop a possible treatment strategy and to use these molecules as new therapeutic agents, further studies are needed in order to obtain more comprehensive data on the mechanisms of action of these molecules and to investigate their possible effects in other cancer types, and the obtained data should be supported by these studies.

Keywords: Prostate cancer, 5-Hydroxy-L-tryptophan, L-tryptophan, MTT assay, LDH release

Introduction

Prostate cancer, which is the most diagnosed cancer among men in Europe and America, is the second most common cause of death from cancer in men. It is observed that mortality rates have decreased due to the widespread use of prostate-specific antigen (PSA) secreted in the prostate, which is thought to contribute to the motility of spermatozoa, and developments in transrectal ultrasound-guided prostate needle biopsy (1–3).

It has long been known that prostate cancer is dependent on androgen for its growth and progression. Androgens, produced in the testicles, adrenal glands, and prostate gland, are essential for normal development and function of the prostate and prostate
cancer proliferation. Therefore, androgen deprivation is an effective therapeutic strategy widely used in clinical practice and has become the standard treatment for this disease. Androgen deprivation therapy (ADT) is used to suppress androgenic effects and therefore prevent progression of prostate cancer (4,5). However, most patients develop resistance to metastatic castration after several years of ADT therapy and progress to prostate cancer (6). In addition, since androgens affect many other organs besides the prostate, according to the mechanism of action of the drug used, ADT may cause decreased libido, erectile dysfunction, hot flashes, loss of bone density, bone fractures, loss of muscle mass and physical strength, changes in blood lipids, insulin resistance, weight gain, it can have various side effects such as burnout and gynecomastia. Today, many new drugs are introduced to the market for use in the treatment of prostate cancer. Due to these limitations and adverse effects of current standard treatments, the search for safer and more effective molecules based on naturally occurring compounds is emerging.

L-Tryptophan is an essential aromatic α-amino acid and is required in the diet of children and adult humans. It serves as a precursor for important biomolecules such as kynurenic acid, nicotinamide adenine dinucleotide, serotonin, melatonin, tryptamine, and niacin in addition to being a necessary amino acid for protein synthesis (7–9). 5-Hydroxy-L-tryptophan (5-HTP), a serotonin pathway metabolite of L-tryptophan in the brain that regulates serotonin levels. As a result, 5-HTP is a key player in the serotonin pathway. Additionally, 5-HTP is a naturally occurring aromatic amino acid that has a variety of antioxidant properties (10–13). Additionally, 5-Hydroxy-L-tryptophan (5-HTP) is a well-known dietary supplement that has taken the place of L-tryptophan (L-Trp) as a treatment for depression (14,15), fibromyalgia’s incapacitating symptoms (16,17), weight loss assistance (18), headache prevention (19), and assistance for insomniacs (20,21). Taking together all these data, the aim of this study was to elucidate the anticancer potentials L-tryptophan and 5-HTP on prostate cancer cells. Therefore, L-tryptophan and 5-HTP were screened for their cytotoxicity using 3-(4,5-dimethyl-thiazol-2-yl) 2,5-diphenyltetrazolium bromide (MTT) assay and LDH release assay. Also, they were examined for antioxidative and oxidative status using total antioxidant capacity (TAC) and total oxidant status (TOS) assay.

Materials and Methods

Cell culture: PC3 and DU-145 cells cell line were kindly provided from Dr. Ömer Faruk Karataş (Erzurum Technical University). The cells were grown in Dulbecco’s Modified Eagle Medium/Nutrient Mixture F-12 (DMEM/F-12) (Gibco, Life Science, USA) (1:1) medium supplemented with 10% fetal bovine serum (PAN Biotech®), 1% streptomycin/penicillin and maintained at 37°C in a 5% CO2 incubator.

MTT Assay: For MTT assay, 1x10⁴–1x10⁵ cells were seeded in 96-well plates and kept under appropriate culture conditions (37°C, 5% CO2) for 24 h for cell attachment. Then, cells were incubated with different concentrations of 5-HTP and L-tryptophan for 48h. After incubation period, MTT solution (5 mg/ml MTT in PBS; Sigma-Aldrich®, Germany) was added and incubated for 3 h. Then, dimethyl sulfoxide (DMSO) (Merck®, Germany) is used to dissolve formazan crystals. In a microplate reader, the optical density was measured at 570 nm of wavelength (Synergy-HT; BioTek Winooski, VT, USA). Cells were given a treatment with 0.1% (w/v) Triton X-100 as a positive control. The untreated cells were used as a negative control. With the use of Probit-log concentration graphs, the IC₅₀ value was calculated.
**LDH Release Assay:** Following the manufacturer's instructions, the LDH test was carried out using the CytoSelect™ LDH Cytotoxicity Assay Kit (Cell BioLabs, San Diego, CA). Briefly, after the above-mentioned treatments, 90 μl of the supernatant from the cells were transferred to a fresh plate, and 10 L of the reaction mixture were applied to each well. The reaction was incubated for 30 minutes at room temperature in the dark. Ultimately, a microplate reader was used to detect the optical density at a wavelength of 450 nm. (Synergy-HT; BioTek Winooski, VT, USA). As a positive control, cells were treated with 0.1% (w/v) Triton X-100. The cells without treatment served as negative control.

**Oxidative analysis:** TAC assay and TOS assay were carried out according to provider's manual. Briefly, the cells were cultured in 96-well plate and treated with 5-HP in a concentration of 95.23 mg/ml for 48 h. At the end of incubation, commercially available TAC and TOS assay kits (Rel Assay Diagnostics®, Turkey) were used according to the manufacturer's instructions to measure antioxidative/oxidative capacity of 5-HP. Ascorbic acid (10 μM) and hydrogen peroxide (25 μM) from Sigma-Aldrich Company were preferred as positive control treatments in determining TAC and TOS levels, respectively.

**Statistical Analysis:** Statistical analysis was conducted using SPSS® 21.0 program. The results are given as mean ± standard deviation. Duncan's test was used as a post-hoc followed by one-way analysis of variance (ANOVA). p < 0.05 was set as the minimal level of significance.

**Results**

Two different cell viability assays were carried out to measure the anti-proliferative effect of 5-HP and L-tryptophan on the human prostate cancer cells in order to obtain more reliable data. Markers of energy metabolism of cell is measured by MTT reduction assay and loss of cell membrane integrity was determined by LDH release assay. The cytotoxicity assays demonstrated that 5-HP led to decreased cell growth depending on dose (p<0.05) (Figure 1).

As a result of the study, IC₅₀ values of 5-HP was determined as 95.23 mg/ml for PC3 and 108.58 mg/ml for Du-145 cells. On the other hand, IC₅₀ values of L-tryptophan was calculated as 543.67 mg/ml for PC3 and 793.12 mg/ml for Du-145 cells. Also, LDH release assay exhibited similar results with MTT assay (Figure 2).

Since 5-HP showed highest cytotoxic activity on PC-3 cells, antioxidative capacity was determined using TAC and TOS assays. Treatments of cells with 5-HP (at IC₂₀ concentration) and positive control agents (as ascorbic acid and hydrogen peroxide) resulted in changes of TAC and TOS levels as compared to untreated cells. 5-HP considerably slightly expanded TAC level (1.9x fold) on PC3 cells while this concentration did not cause a change in TOS level. (Table 1).

**Discussion**

One of the most used criteria for cytotoxicity is cell membrane integrity, and many approaches have been established for its evaluation (22). Numerous investigations have employed the release of stable intracellular enzymes like lactate dehydrogenase (LDH1 as well as the release of a label like 51Cr or calcein (23–26). Other options for assessing general cytotoxicity include tests that evaluate cell metabolic activity. For instance, many tetrazolium compounds have been utilized for this (27,28). In the present study, we assessed MTT and LDH release assay and both results showed a dose-dependent reduction in viability of both prostate cancer cells after 48 h incubation with 5-HP.
Figure 1. Effects of 5-HTP and L-tryptophan (0-800 mg/ml) on viability of PC-3 (A) and Du-145 (B) cells. Data represented as mean ± SEM (*p <0.05).

Figure 2. Effects of 5-HTP and L-tryptophan (0-800 mg/ml) on LDH activity of PC-3 (A) and Du-145 (B) cells. Data represented as mean ± SEM (*p <0.05).
Oxidative stress is a factor in the development of a number of diseases and is brought on by the interaction of chemically reactive oxygen species with biomolecules. Angiogenesis, uncontrolled proliferation, apoptosis escape, uncontrolled proliferation, tissue invasion, and metastasis are all associated with oxidative stress at different phases of cancer development (29–31). As a result, substances having antioxidant properties may play a crucial role in chemoprevention by lowering oxidative stress. Because of their low molecular weight, simple absorption, and great action, researchers have recently focused a lot of attention on antioxidative peptides. It has been observed that several amino acids and their derivatives, including cysteine, histidine, tryptophan, lysine, arginine, leucine, valine, and 5-hydroxytryptophan, have antioxidant properties. (32–35). A naturally occurring amino acid containing amino and hydroxy groups, 5-hydroxytryptophan serves as a metabolic key component for the production of the neurotransmitters melatonin and serotonin. The powerful ability of melatonin to scavenge free radicals is influenced by 5-hydroxytryptophan. (36,37). Various tissues have been shown to be protected against oxidative stress by indoles, both natural and synthetic, mostly by scavenging harmful reactive oxygen species (37–39). Several researches have discovered that substituted indoles have antioxidant properties (40–42).

Notably, we found a significant reduction in TOS level and an increased TAC level in 5-HTP treated cells. These results support the antiproliferative potential of 5-HTP as a chemotherapeutic agent in cancer treatment. Collectively, it can be suggested that 5-HTP may have a good potential for anticancer activity in prostate cancer via decreasing oxidative stress and promoting cell death. However, further investigations are still needed to understand the precise mechanism of the anticarcinogenic effect of 5-HTP in prostate cancer.

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**References**


[34] Padillo FJ, Cruz A, Navarrete C, Bujalance I, Briceño J, Gallardo

